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The nerve growth factor/tumor necrosis factor receptor family

Martin Lotz, Morey Setareh, Johannes von Kempis, and Herbert Schwarz

Department of Medicine, School of Medicine, University of California, San Diego

Abstract: Receptors in the nerve growth factor/tumor necrosis factor receptor family are characterized by the presence of cysteine-rich motifs of ~40 amino acids in the extracellular domain. The ligands are type II transmembrane proteins with β-strands that form a jelly-roll β-sandwich. The receptors recognize soluble or cell-surface-bound ligands and mediate diverse cellular responses. Activation of intracellular signals is mediated at least in part by the association of proteins with a RING finger motif or a death domain to the cytoplasmic domains of the receptors. In addition to cell-membrane-bound receptors soluble forms have been described for most of the receptors. Activation of intracellular signals not only occurs through ligand binding to the receptors but cross-linking of at least some members of the ligand family can regulate cell functions. *J. Leukoc. Biol.* 60: 1–7; 1996.

Key Words: host defense · differentiation · cell proliferation · apoptosis

INTRODUCTION

Members of the nerve growth factor/tumor necrosis factor (NGF/TNF) receptor family are characterized by the presence of one to six cysteine-rich motifs of approximately 40 amino acids in the extracellular domain. The cysteine-rich regions provide the motif for binding to shared structures in the ligands [1, 2]. The receptor family now includes the low-affinity nerve growth factor receptor (NGFR) [3], TNFR1 (or TNFR55) [4, 5], TNFR2 (or TNFR75) [6], the TNF receptor-related protein (TNFRrp) [7], which is a Lymphotoxin (LT) β-specific receptor [8], CD40 [9], the Hodgkin's antigen CD30 [10], the T cell antigen CD27 [11], Fas/APO-1 [12, 13], OX-40 [14], and 4-1BB/ILA [15, 16]. Shope fibroma virus, cowpox virus, myxoma viruses, and vaccinia viruses contain genes that are probably acquired from the host cellular genome that encode soluble TNF receptors. The proteins are secreted from virus-infected cells, bind TNF and LT, and inhibit their biological activity [17]. The TNF ligand family includes TNF, LTα (also referred to as TNF-β), LTβ [18], the CD40 ligand gp39 [19], CD70, the CD27 ligand [20], Fas ligand [21], 4-1BB ligand [22], and OX-40 ligand [23]. The TNF ligand superfamily members, with the exception of LTα, are type II membrane glycoproteins with homology to TNF in the extracellular domain.

TNF and Fas regulate function of a broad spectrum of cell types and are implicated in diverse aspects of host defense responses and the pathogenesis of different diseases. Other members of the family, such as CD27, CD30, CD40, OX-40, and ILA/4-1BB appear to be involved primarily with the regulation of immune responses. This review will summarize structure of receptors and ligands, signal transduction, and major biological functions.

STRUCTURE OF RECEPTORS AND LIGANDS

The structure of TNFR1 extracellular domain has been determined on the basis of crystallization in complex with TNF-β [24] as well as in the absence of ligand [25]. The TNFR/TNF-β complex consists of three receptor molecules that are symmetrically bound to one TNF-β trimer. The receptor is an elongate molecule with four disulfide-rich domains in a nearly linear array and binds in the groove between two adjacent TNF-β subunits. The unliganded domains can form dimers of two distinct types [25]. Antiparallel associations occur through an interface that overlaps the TNF binding site. This form of association would separate the cytoplasmic domains and could inhibit signaling in the absence of TNF. Parallel dimers are also observed in which the dimer interface is well separated from the TNF binding site. Associations among TNF-bound parallel dimers could cause receptor clustering.

In addition to cell-membrane-bound receptors within this family soluble forms have been described for the low-affinity NGFR [26], TNFR1, and TNFR2 [27, 28], Fas [29], CD27 [30], CD30 [31], CD40 [32], and 4-1BB [33] (**Table 1**). Soluble TNFR appear to be generated by proteolytic cleavage of the membrane-associated forms because for each of these receptors only a single mRNA species has been detected. This is in contrast to the soluble form of the Fas molecule, which originates from an RNA splice variant [29]. The soluble form of Fas was also biologically active and inhibited apoptosis induced by an agonistic antibody. A mRNA splice variant of murine 4-1BB lacking the coding region for the transmembrane domain was detected in different tissues [33]. However, a

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Abbreviations: TNF, tumor necrosis factor; TNR-R, TNF receptor; NGF, nerve growth factor; LT, lymphotoxin; TRAF, TNF receptor-associated factor; SLE, systemic lupus erythematosus; NF-κB, nuclear factor-κB; Ig, immunoglobulin.

Reprint requests: Martin Lotz, UCSD, La Jolla, CA 92093-0663.

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TABLE 1. Soluble Forms of Receptors and Ligands

Receptor	Soluble form	Ligand	Soluble form
TNFR1	Cleavage	TNF	Cleavage
TNFR2	Cleavage	TNF, LTα	Cleavage
LTβR	ND	LTβ	ND
Fas	Alternate splice	FasL	ND
CD27	Cleavage	CD27L/CD70	ND
CD30	Cleavage	CD30L	ND
CD40	Cleavage	CD40L/gp39	Intracellular processing
OX-40	ND	OX-40L	ND
4-1BB	Alternate splice	4-BBL	ND
NGFR	Cleavage		

ND, not demonstrated.

similar mRNA encoding a soluble form of the human homologue ILA was not detected in an analysis of a broad spectrum of cell types. Recombinant forms of the soluble receptors that contain the entire extracellular regions or parts thereof have been important tools in characterizing the biological functions of the TNF-R and are under investigation as therapeutic agents in sepsis, arthritis, and other conditions [34].

The ligands of the TNF family are type II transmembrane proteins. The structures of TNF and LTα have been determined by X-ray crystallography [35–37]. The monomers represent eight β-strands that form a jelly-roll β-sandwich motif. Threefold related subunits form a trimer stabilized primarily by hydrophobic interactions. TNF has three sites at the interface between the subunits that can interact with the receptor. Based on modeling studies, the structures of the other ligands appear to be similar to TNF.

SIGNAL TRANSDUCTION THROUGH RECEPTORS

Activation of the TNF receptors is triggered by the aggregation of cytoplasmic domains that occurs when the extracellular domains of two or three receptors bind to trimeric TNF or LTα.

Several downstream signaling events that are activated by TNF and other ligands of the cytokine family had been characterized [38] but signaling molecules that mediate the initial interaction with the ligand-occupied receptor have only recently begun to be identified. The method used in most of these studies for the isolation of molecules that interact with the intracellular domains of the receptors was the yeast two hybrid system.

A region of 76 amino acids was identified by mutational analysis to be required for signal transduction by the TNFR2. When this region was used in affinity purification and in the yeast two hybrid system, two novel proteins, termed TNF receptor-associated factors (TRAF), were isolated. TRAF1 showed no significant sequence similarity to previously known molecules. TRAF2 contained an N-terminal RING finger sequence motif that may form zinc binding structures mediating protein-DNA or possibly protein-protein interactions. TRAF1 and TRAF2 contain a highly homologous region of 230 amino acids, called TRAF domain. This region appears to mediate heterodimer formation. Because TRAF1 showed only weak direct binding to TNF-R2, it has been suggested that TRAF2 interaction with TNF-R2 allows association of TRAF1 [39]. A protein that also contains a C-terminal TRAF domain and an N-terminal RING finger motif was identified on the basis of its interaction with the cytoplasmic domain of CD40 [40]. This molecule, termed TRAF3, also binds to an Epstein-Barr virus-encoded protein and is probably involved in signaling events leading to Epstein-Barr virus-induced B cell transformation [41]. TRAF3 self-associates but does not dimerize with TRAF1 or TRAF2 [42]. There also appears to be selectivity and specificity in the interaction of the TRAFs with the different members of the TNF receptor family. TRAF1–3 do not bind to Fas or TNFR1, TRAF1 does not interact with CD40, and TRAF3 does not bind to TNFR2 via the TRAF domain in the yeast two hybrid system [42] but it co-immunoprecipitates with TNFR2 [41]. Furthermore, with respect to downstream signaling events, there are differences. TRAF2 but not TRAF1 or TRAF3 mediates nuclear factor-κB (NF-κB) activation by TNFR2 and CD40. NF-κB activation is dependent on the presence of the RING finger motif [42].

CD40 activation on B cells inhibits programmed cell death. A zinc finger protein, A20, is induced by the Epstein-Barr virus LMP-1 gene product and inhibits B-cell apoptosis. CD40 activation induces A20 by inducible binding of NF-κB complexes to the A20 promoter [43].

The intracellular domain of Fas contains a sequence, termed death domain, that is required for the induction of apoptosis. This sequence motif is also present in TNFR1, CD40, and NGFR. Three proteins that are unrelated to the TRAF family and bind to death domains have been identified: TNFR1-associated death domain protein, TRADD, which mediates NF-κB activation and apoptosis by TNFR1 [44]. FADD and RIP bind to the death domain in Fas and, on overexpression, induce apoptosis. All three proteins contain death domains that are similar to those in the TNFR1 and in Fas and mediate interactions with the receptors.

Fas also contains a 15-amino acid C-terminal motif that functions as a negative regulatory domain that can suppress Fas-generated signals leading to apoptosis. A protein tyrosine phosphatase, termed FAP-1, interacts with this motif and the high levels of its expression correlates with the resistance to Fas-mediated cytotoxicity [45].

SIGNAL TRANSDUCTION THROUGH MEMBRANE-ASSOCIATED LIGANDS

Signal transduction occurs not only through the receptors but recent evidence suggests that cross-linking of the membrane-associated CD40L, OX-40L, and ILA/4-1BB ligand can trigger intracellular signals and regulate cell functions. The quality of a signal varies with target cell or

TABLE 2. Consequences of Genetic Defects in Receptors or Ligands

Gene	Phenotype	Reference
Spntaneous mutation		
CD40L (human)	Hyper IgM syndrome (eleated IgM) virtual absense of other isotypes	[91]
Fas (mouse)	Lymphadenopathy; autoimmune manifestations	[60]
FasL (mouse)	Similar phenotype as in Fas mutation	[47]
Knock out		
TNFR1	Resistance to LPS-induced lethality; defect in clearing *Listeria monocytogenes* infection	[55, 56]
TNFR2	Resistance to TNF-induced death and tissue necrosis	[57]
CD40	Defect in T cell dependent antibody production and isotype switching; absence of IgE	[81]
CD40L	Similar phenotype as CD40 knock out	[82]
LTα	Abnormal development of peripheral lymphoid organs	[59]

activation state. The ligand and receptor can induce qualitatively opposing effects on the same cellular response.

CD40L is expressed on activated but not on resting T lymphocytes. Much higher levels of CD40L are expressed on $CD4^+$ compared with $CD8^+$ cells. CD40 expressed on transfected cells enhanced anti-CD3-induced proliferation of $CD4^+$ cells but had only marginal effects on $CD8^+$ cells [46].

OX-40L is expressed on activated T and B cells. Cross-linking of OX-40L enhanced proliferation of B cells, immunoglobulin heavy chain mRNA levels, and immunoglobulin secretion. Cross-linking of OX-40 ligand also altered the levels of the transcription factor BSAP, thus providing direct evidence for signal transduction through this ligand [47].

Antibodies to ILA/4-1BB co-stimulate lymphocyte proliferation. However, fusion proteins containing the extracellular part of ILA/4-1BB inhibit T cell proliferation and induce cell death [48]. These effects are observed only when the fusion proteins are fixed but not in solution, suggesting that cross-linking of the ligand is required for the induction of the cellular response. This example also illustrates that the same receptor-ligand pair can induce cell functions in both receptor as well as ligand-expressing cells and that the cellular responses are qualitatively distinct, representing increased proliferation and the induction of cell death, respectively. This pattern of signaling allows a novel form of communication during cell-cell interactions. The significance of this mechanism for example in the interaction of antigen-presenting cells and lymphocytes has not yet been fully explored.

BIOLOGICAL FUNCTIONS

Biological functions of receptors and ligands in this family have extensively been reviewed elsewhere [49–53]. Here we will briefly summarize the major roles of the receptors by focusing on genetic evidence in characterizing function. Initial functional characterization for the receptors was performed with ligands, soluble receptors, and antibodies. More recently, fusion proteins that contain the extracellular part of a receptor and the constant domain of immunoglobulin G have been used in different in vitro and in vivo models. Several spontaneous mutations in the receptor genes and deletions by homologous recombination have been described (**Table 2**).

TNF and LT

TNF can induce a broad spectrum of biological effects such as cell death, gene induction, antiviral activity, and cytokine production. The TNF ligand family now includes TNF, LTα, and LTβ. In addition to cell-membrane-bound ligands within this family, soluble forms are known to occur naturally for TNF, which is synthesized as a 26-kDa precursor protein. This is processed to a secreted 17-kDa mature form by a unique Zn^{2+} endopeptidase, also termed TNF convertase [54]. The cell surface form of LTα is assembled during biosynthesis as a heteromeric complex with LTβ, a type II transmembrane protein [18]. Secreted LTα is a homotrimer that binds to distinct TNF receptors of 60 and 80 kDa. However, these receptors do not recognize the major cell surface LTα-LTβ complex. A receptor specific for human LT-β was identified, which suggests that cell surface LT may have functions that are distinct from those of secreted LTα [8]. Gene targeting of TNFR1 confirmed its role in the lethality in response to low doses of lipopolysaccharide after sensitization with D-galactosamine but the toxicity of high doses of lipopolysaccharide was unaffected. TNFR1 mutant mice were severely impaired in their ability to clear infection with the facultative intracellular bacterium *Listeria monocytogenes* [55, 56]. TNFR2-deficient mice show normal T cell development and activity but have increased resistance to TNF-induced death and a decrease in TNF-induced tissue necrosis [57]. Studies on fibroblasts from TNFR1-deficient mice suggested that this receptor controls adhesion to leukocyte cell lines as well as ICAM-1, VCAM-1, CD44, and MHC class I up-regulation, secretion of other cytokines, cell proliferation, and NF-κB activation. Stimulation through TNFR2, in TNFR1-deficient fibroblasts, did not have any effect in these functions [58].

Mice deficient in LTα by gene targeting have no morphologically detectable lymph nodes or Peyer's patches, although development of the thymus appears normal. Within the white pulp of the spleen there is failure of normal segregation of B and T cells. Spleen and peripheral

blood contain $CD4^+/CD8^-$ and $CD4^-/CD8^+$ T cells in a normal ratio, and both T cells subsets have an apparently normal lytic function. Lymphocytes positive for immunoglobulin M are present in increased numbers in both the spleen and peripheral blood. Thus, LTα appears essential in the normal development of peripheral lymphoid organs [59].

Fas/APO-1

Fas, also termed APO-1, was discovered as a cell membrane receptor, which, upon activation by specific antibody, triggered cell death by apoptosis. The lymphoproliferation (*lpr*) mutation in the MRL strain of mice is caused by the insertion of a transposable element in the Fas gene. The insertion causes a decrease in Fas mRNA expression and the Fas protein is not expressed on resting or activated lymphocytes from MRL lpr/lpr mice. These findings suggest that Fas plays a role in both thymic selection and T cell survival in the periphery and that the accelerated autoimmunity in MRL lpr/lpr mice results from a defect in both of these pathways [60].

Recombinant Fas ligand induced apoptosis in Fas-expressing target cells. Fas ligand is expressed in activated splenocytes and thymocytes, consistent with its involvement in T cell-mediated cytotoxicity and in several nonlymphoid tissues [21]. The MRL mouse strain with generalized lymphadenopathy (*gld*) develops similar autoimmune manifestations as the *lpr* strain. The gld mutation is a point mutation in the Fas ligand that abolishes binding to the receptor [61].

A potential association of an impairment in the induction of apoptosis and human systemic lupus erythematosus (SLE) has been suggested. Peripheral blood mononuclear cells from SLE patients produced increased levels of a soluble form of Fas. This receptor competes for binding of Fas ligand and protects cells from apoptosis [29]. Possible consequences could be a persistence of autoreactive lymphocytes or the release of undegraded DNA from necrotic cells, which could stimulate the formation of anti-DNA antibodies.

CD27

CD27 is a transmembrane homodimer with subunits of 50–55 kDa expressed only on lymphoid cells, including the majority of peripheral T cells, a subset of B cells, NK cells, and CD3 bright thymocytes. During lymphocyte activation the expression of CD27 increases and a soluble 28- to 32-kDa form of CD27 (sCD27). One mRNA encodes both the transmembrane receptor and sCD27. The transmembrane form gives rise to sCD27 most likely via a proteolytic event [62]. sCD27 has been detected in body fluids from healthy individuals [30, 52].

CD27 co-stimulates proliferation and enhances cytokine synthesis of T cells that are activated by mitogens, antigens, or antibodies to CD2, CD3, or CD28 [63, 64]. This receptor is also involved in the PWM-driven T cell-dependent IgG synthesis [64].

CD27 is expressed on most but not all peripheral blood $CD4^+$ T cells. The small fraction of $CD4^+$ T cells with a $CD27^-$ phenotype exclusively resides within the $CD45RA^-CD45RO^+$ subset. $CD27^-$ cells are functionally differentiated cells that have lost CD27 expression as a result of persistent antigenic stimulation. $CD27^+$ and $CD27^-$ cells do not differ notably in the expression of CD70 (CD27 ligand) [65, 66]. CD27 is also expressed on a subpopulation of human B lymphocytes and positively correlated with membrane immunoglobulin (Ig) A but negatively correlated with membrane IgM/membrane IgD positivity. CD27 on B cells can be induced selectively by the combination of *Staphylococcus aureus* plus interleukin-2. After in vitro stimulation, $CD27^+$ but not $CD27^-$ B cells secrete large amounts of both IgM and IgG. CD27 may thus be a marker that discriminates naive from primed B lymphocytes [67].

These functions of CD27 on lymphocytes were confirmed with CD27 ligand. Cloned CD27 ligand co-stimulated T cell proliferation and enhanced the generation of cytolytic T cells [68], cytokine production, induction of activation antigens, and proliferation of unprimed $CD45RA^+$, and to a lesser extent, of primed $CD45RO^+$ peripheral blood T cells [69]. CD27L is identical to the previously identified activation antigen CD70. CD70 expression in vivo is confined to activated B and T lymphocytes [70]. On T cells, CD70 was expressed almost equally on both activated CD4 and CD8 cells. On subsets of CD4 T cells, however, CD70 expression was induced preferentially on the CD45RO T cell population after activation, whereas its expression was not seen on CD45RA T cells [71].

CD30

CD30 was originally described as a cell-surface antigen on primary and cultured Hodgkin's and Reed-Sternberg cells [53]. CD30 is normally expressed by a subset (15–20%) of $CD45RO^+$ T cells after activation by a variety of T cell stimuli. $CD30^+$ T cells are preferentially regulated by IL-12, and the effects of IL-12 on T cell IFN-γ production are mediated largely through its effects on the $CD30^+$ subset. $CD30^+$ T cells also secreted higher levels of IL-5 than activated $CD30^-$ T cells. In contrast $CD30^-$ T cells produced significantly higher levels of IL-2 than $CD30^+$ T cells. $CD30^+/CD4^+$ T cells exhibit significantly greater helper activity for B cell Ig production than $CD30^-/CD4^+$ T cells. Thus, $CD30^+$ T cells are the major interferon-γ- and interleukin-5-producing T cells, and exhibit potent helper activity for Ig production [72, 73].

Soluble CD30 is released by T cell clones and tumor cells. High serum levels of sCD30 were observed in atopy, SLE, and after infection with measles virus or human immunodeficiency virus [74].

Recombinant human CD30 ligand enhanced Ig secretion of Epstein-Barrvirus-transformed B-cell lines and increased proliferation of some tumor cells, whereas in others it induced cytolytic cell death [75]. CD30 is expressed

constitutively on the human T cell line ACH-2, which is chronically infected with HIV-1. Cross-linking CD30 results in HIV expression, which is associated with NF-κB activation and enhanced HIV transcription [76].

CD40

CD40 is expressed on B lymphocytes, thymic epithelial cells, activated monocytes, dendritic cells, hematopoietic progenitor cells, epithelial cells, and carcinomas. Cross-linking of CD40 with immobilized anti-CD40 or cells expressing CD40L induces high levels of B cell proliferation and addition of IL-4 or IL-13 allows the generation of factor-dependent long-term normal human B cell lines and the secretion of IgE following isotype switching [77].

CD40 ligand (CD40L), a 39-kDa glycoprotein, is transiently expressed on activated T cells, mostly $CD4^+$ but also some $CD8^+$ as well as basophils and mast cells. Soluble CD40L is an 18-kDa protein that is generated by intracellular processing [78, 79]. Individuals with X-linked hyper-IgM syndrome fail to express functional CD40L and, as a consequence, are incapable of mounting protective antibody responses to opportunistic bacterial infections [80].

In CD40 and RAG-2 knock-out mice where all mature lymphocytes were derived from the CD40-deficient embryonic stem cells, T and B cell number and phenotype were normal. However, $CD40^{-/-}$ chimeras completely failed to mount an antigen-specific antibody response or to develop germinal centers following immunization with a T cell-dependent antigen but responded normally to T cell-independent antigens. The $CD40^{-/-}$ animals had an absence of IgE and a severe decrease of IgG1 and IgG2a [81]. Similar results were obtained with mice deficient in CD40L expression [82]. These results support the essential role of CD40- CD40L interactions for T cell-dependent antibody responses and in isotype switching and show that Ig class switching to isotypes other than IgE can occur in vivo in the absence of CD40L. CD40 also mediates various functional effects on other cell types [83].

A role in the pathogenesis of collagen-induced arthritis has been suggested by studies where administration of gp39 antibodies reduced disease severity and decreased the titers of antibodies to type II collagen [67].

OX-40

OX-40 expression appears to be restricted to activated T cells [84] where it acts as a costimulatory receptor. Cloning of the ACT35 lymphocyte activation antigen revealed that it corresponds to human OX-40 [85].

Human OX-40 ligand, gp34, was previously known to be expressed by T cell lymphotropic virus 1-infected cells. Recombinant OX-40 ligand expressed in COS cells costimulates phorbol myristate acetate, phytohemagglutinin, and anti-CD3-induced $CD4^+$ T cell proliferation [23].

Expression of OX-40L was detected on activated T cells, with higher levels found on $CD4^+$ than $CD8^+$ cells [86]. OX-40 ligand was also expressed on a subset of peritoneal B cells and LPS-activated splenic B cells [84]. Cell-bound recombinant ligands co-stimulate T cell proliferation and cytokine production, in particular IL-4 secretion [86].

4-1BB/ILA

4-1BB was initially identified as a gene that is inducibly expressed by murine T lymphocytes [15, 87]. Cross-linking of 4-1BB enhanced anti-CD3-induced T cell proliferation [88] as well as the proliferation of anti-u-primed splenic B cells [89]. ILA, the human homologue of 4-1BB, was also discovered as a gene that is expressed in activated T cells [16]. In addition to T cells, ILA is also expressed on B lymphocytes, monocytes, epithelial cells, and chondrocytes [90]. Expression in all of these cell types is activation-dependent. Antibodies to ILA co-stimulated anti-CD3-induced proliferation of human T lymphocytes. However, when anti-CD3-stimulated T cells were cultured in the presence ILA-IgG fusion protein in solid-phase, it inhibited proliferation and induced apoptosis [48]. This was not observed with soluble fusion protein, suggesting the possibility that 4-1BB ligand is capable of providing an anti-proliferative or death-inducing signal to the cells.

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